学術論文

Effects of the Feeding of Soybeans on Serum and Liver Lipid Levels in Rats: Comparison of Effects between Yellow and Black Soybeans[*1]

ラットの血清および肝臓脂質レベルに対する大豆摂取の影響 －黄大豆と黒大豆の効果の比較－

Ayaka OBARA,[*2,3] Tomoka SATO,[*2,3] Asuka FUGANE,[*2,3] Erina YACHI[*2,3] and Masashi KAWASAKI[*2,4]
小原亜矢香，佐藤友花，府金明日香，谷地永里菜，川崎雅志

The effects of the feeding of soybeans on serum and liver lipid levels were studied in rats. Rats were fed an experimental diet containing yellow or black soybeans and containing the same levels of protein, lipids and dietary fibers as the basal diet. The serum total cholesterol concentration in the yellow soybean diet group was significantly lower than that in the basal diet group. As for the lipoprotein cholesterol concentrations, the HDL-cholesterol concentration in the yellow soybean diet group was significantly lower than that in the basal diet group, whereas there were no significant differences in the VLDL-cholesterol and LDL-cholesterol concentrations among the three diet groups. The liver cholesterol content was not significantly different among the three diet groups, although that in the yellow and black soybean diet groups was lower but without a significant difference as compared to the basal diet group. The serum and liver triglyceride levels in the yellow and black soybean diet groups were significantly lower than those in the basal diet group. The liver TBARS value in the yellow and black soybean diet groups was significantly higher than that in the basal diet group, while that in the black soybean diet group was significantly lower than that in the yellow soybean diet group. These results suggest that the feeding of both yellow and black soybeans has beneficial effects on lipid metabolism; thus, it is recommended to incorporate these soybeans into the daily diet.

Keywords: *liver lipid, serum lipid, soybeans*
肝臓脂質，血清脂質，大豆

INTRODUCTION

Soybeans are a common staple food throughout the world. In Japan, the consumption of soybeans is very high, and many processed foods are produced from soybeans. *Miso*, soy sauce, and *natto*, which are well-known Japanese traditional foods, are made by fermenting soybeans. *Tofu* is made by coagulating soy milk and pressing the resulting curds.

The nutritional value of soybeans is high among vegetables. Soybeans are rich in protein that is especially rich in essential amino acids as compared to other plant proteins, with an amino acid score of 100.[1)] In terms of fatty acids, soybeans are rich in linoleic acid and oleic acid,[2)] and in terms of phospholipids, soybeans are rich in lecithin. Linoleic acid is an essential fatty acid and is a precursor of arachidonic acid and dihomo-γ-linoleic acid, which are the precursors of eicosanoids. Lecithin makes up the cell membranes as well as brain and nerve tissue. Soybeans are rich in vitamins B_1 and B_2, and *natto* is especially rich in vitamin K.[3)]

It is well known that soybeans have many nutritional functions. For example, soybean protein reduces the serum cholesterol concentration.[4-7)] The soy isoflavones, genistein and daidzein, are the two major isoflavones and have structural similarities to 17β-estradiol, exhibiting weakly estrogenic action by binding to estrogen receptors. They have attracted wide attention because of their potential beneficial effects in preventing menopausal symptoms,[8)] osteoporosis,[9)] cardiovascular diseases[10)] and cancers.[11)]

Some kinds of soybeans have a black seed coat that contains anthocyanin. Anthocyanin is a water-soluble pigment that may appear red under acidic conditions or purple and blue under alkaline conditions. Anthocyanins occur in the leaves, stems, roots, flowers, and fruits of plants. Anthocyanin is one kind of polyphenol and, among other polyphenols, it is well known to have an antioxidant function.[12)]

The present study examined the effects of the feeding of soybeans on the serum and liver lipid levels in rats. Rats were fed an experimental diet containing soybeans with different seed coat colors, i.e., yellow and black, and containing the same levels of protein, lipid and dietary fibers.

MATERIALS AND METHODS

Animals and diets. This animal experiment was conducted with the approval of the Iwate Prefectural University Research Ethics

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*1 This study was conducted as a part of the Special Training of Food and Nutrition Major, Science of Living Department.
*2 Food and Nutrition Major, Science of Living Department.
*3 Co-first authors. These authors contributed equally to this work.
*4 Corresponding author.
Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; NEFA, nonesterified fatty acid; TBARS, thiobarbituric acid-reactive substance; VLDL, very-low-density lipoprotein.

Table 1. Composition of experimental diets. (g/100g)

Ingredients	Control	Yellow soybean	Black soybean
Casein [1]	20	-	-
α-Cornstarch [1]	13.2	13.2	13.2
Cornstarch [1]	31.25	15.35	15.35
Sucrose [2]	10	10	10
Cellulose powder [1]	9.7	-	-
Corn oil [1]	10.8	-	-
Mineral mixture (AIN93G composition) [1]	3.5	3.5	3.5
Vitamin mixture (AIN93 composition) [1]	1	1	1
Choline bitartrate [3]	0.25	0.25	0.25
L-Cystine [3]	0.3	-	-
Yellow soybean	-	56.7	-
Black soybean	-	-	56.7

[1] Oriental Yeast Co., Ltd., Tokyo, Japan.
[2] Nissin Sugar Manufacturing Co., Ltd., Tokyo, Japan.
[3] Wako Pure Chemical Industries, Ltd., Osaka, Japan.

Committee.

Male Wistar rats (3-wk-old, Charles River Laboratories Japan, Inc., Kanagawa, Japan) were individually housed in stainless steel cages with wire bottoms in an air-conditioned room at a temperature of 22±2℃, relative humidity of 60±5%, and a 12-h light cycle (0800-2000). They were fed a stock pellet diet (MF; Oriental Yeast Co., Ltd., Tokyo, Japan) followed by a basal diet for 4 d. Subsequently, the rats were divided into three groups (n=5) with similar body weights and were fed the experimental diets as follows: The first group was fed a diet containing yellow soybeans (*Suzuhonoka*); the second group was fed a diet containing black soybeans (*Kurosengoku*); and the last group was fed the basal diet. The soybeans were ground. The compositions of the experimental diets[13)] are shown in Table 1. The composition of the soybeans was as follows: 12.5% water, 35.3% protein, 19.0% lipid, 28.2% carbohydrate, 5.0% ash, and 17.1% dietary fibers.[3)] In the control group, corn oil was used instead of soybean oil in order to examine the effect of soybeans or their ingredients in the soybean diet groups. All of the diets contained the same levels of protein (20%), lipid (10.8%) and dietary fibers (9.7%). The rats were kept for 28 d in total. The experimental diet and water were available at all times. Animals were deprived of their diets at 0900 on the 28th day but allowed free access to water until they were sacrificed, which occurred 4 h later. Blood was collected from the heart and left to clot at room temperature so that serum could be obtained. The liver and epididymal adipose tissue were quickly removed, washed with cold 0.9% NaCl, blotted on filter paper, and weighed. The serum and liver were stored at -30℃ until lipid concentration analyses were performed. Aliquots of the liver were also preserved in methanol and stored at 4℃ until analyses of the lipid content were performed.

Lipid analyses. The lipoprotein separation of serum was performed as follows. HDL was separated from VLDL plus LDL by the precipitation method using sodium phosphotungstic acid and MgCl,[14)] and VLDL was separated from LDL plus HDL by ultracentrifugation.[15)]

Serum total, HDL-, and (HDL+LDL)-cholesterol; triglyceride; phospholipid; and nonesterified fatty acid (NEFA) concentrations were determined by an enzymatic method using a Cholesterol E-test, Triglyceride E-test, Phospholipid C-test, and NEFA C-test (Wako Pure Chemical Industries, Ltd., Osaka, Japan), respectively. The difference between the total cholesterol concentration and the HDL-cholesterol concentration was regarded as the (VLDL+LDL)-cholesterol concentration. The difference between the (HDL+LDL)-cholesterol concentration and the HDL-cholesterol concentration was regarded as the LDL-cholesterol concentration. The difference between the (VLDL+LDL)-cholesterol concentration and the LDL-cholesterol concentration was regarded as the VLDL-cholesterol concentration. The ratio of the (VLDL+LDL)-cholesterol concentration to the HDL-cholesterol concentration is designated as the atherogenic index.

Total lipids from the liver were extracted according to the procedure described by Folch *et al.*[16)] After portions of the chloroform phase had been dried under nitrogen, cholesterol,[17)] triglyceride,[18)] and phospholipid[19)] contents were determined.

The serum and liver thiobarbituric acid-reactive substance (TBARS) values were measured according to the method described by Yagi[20)] and Mihara *et al.*,[21)] respectively.

Statistical analyses. Results were expressed as mean ± standard error. Statistical analysis was carried out by one-way analysis of variance followed by Fisher's protected least significant difference (PLSD) test using the SPSS Statistics, version 22 (IBM Japan, Ltd., Tokyo, Japan). A significance level of $p < 0.05$ was used for all the

Table 2. Initial body weight, food intake, body weight gain, and liver and epididymal adipose tissue weights in rats fed different kinds of soybeans.

Measurement	Control	Yellow soybean	Black soybean
Initial body weight (g)	80.0 ± 2.6	80.0 ± 1.6	80.0 ± 1.9
Food intake (g/28d)	648.3 ± 15.2	615.3 ± 23.4	625.1 ± 37.2
Body weight gain (g/28d)	245.9 ± 8.9 [a]	174.0 ± 5.8 [b]	159.4 ± 11.0 [b]
Liver weight (g/100g of body wt)	4.62 ± 0.25 [a]	3.99 ± 0.24 [ab]	3.74 ± 0.12 [b]
Epididymal adipose tissue weight (g/100g of body wt)	1.55 ± 0.10 [a]	1.09 ± 0.10 [b]	1.35 ± 0.20 [ab]

Values represent the means ± standard errors for five rats. Values not sharing a common letter are significantly different at $p < 0.05$ by one-way analysis of variance followed by Fisher's protected least significant difference (PLSD) test.

comparisons.

RESULTS

Table 2 shows the initial body weight, food intake, and body weight gain for the duration of the 28 d of experimental feeding as well as the relative weights of the liver and epididymal adipose tissue at the end of the experimental feeding period. The food intake was not significantly different among the three groups. On the other hand, the body weight gain in the yellow and black soybean diet groups was significantly lower than that in the basal diet group. The liver weight in the black soybean diet group was significantly lower than that in the basal diet group, and the epididymal adipose tissue weight in the yellow soybean diet group was significantly lower than that in the basal diet group.

Serum cholesterol concentrations are shown in Fig. 1. The serum total cholesterol concentration in the yellow soybean diet group was significantly lower than that in the basal diet group. As for the lipoprotein cholesterol concentrations, the HDL-cholesterol concentration in the yellow soybean diet group was significantly lower than that in the basal diet group, whereas there were no significant differences in the VLDL-cholesterol and LDL-cholesterol concentrations among the three diet groups. The atherogenic index was not significantly different among the three diet groups.

Fig. 2 shows the serum triglyceride, phospholipid, and NEFA concentrations. The serum triglyceride concentration in the yellow and black soybean diet groups was significantly lower than that in the basal diet group. The serum phospholipid concentration in the yellow and

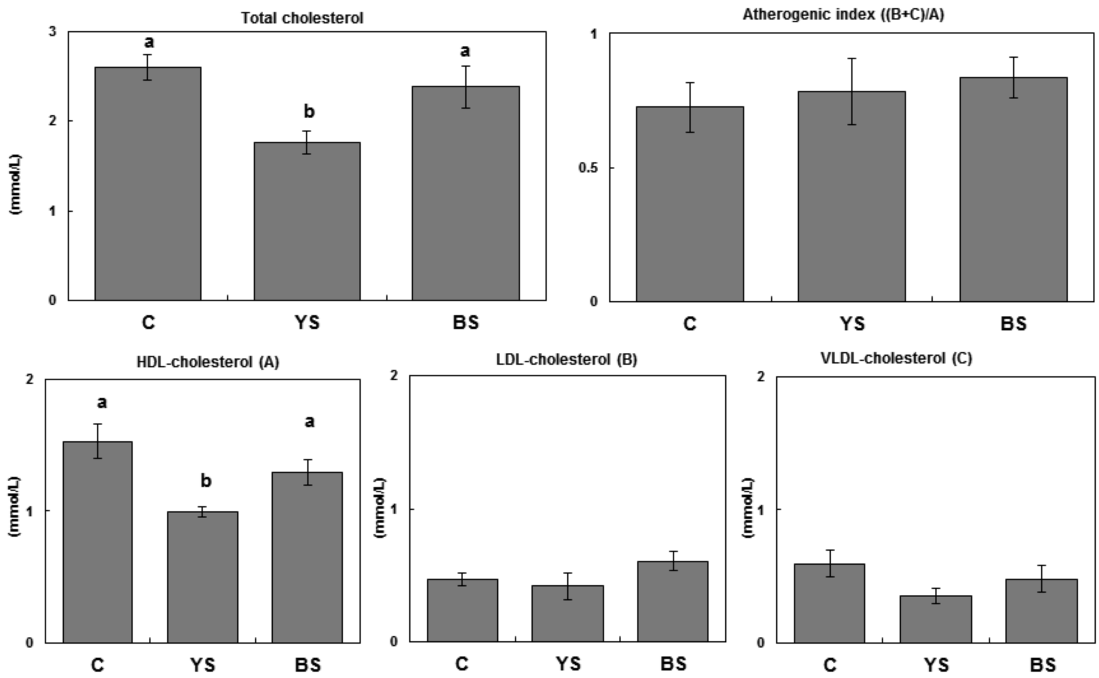

Fig. 1. Effects of the feeding of soybean on serum cholesterol concentration and atherogenic index in rats. Values represent the means for five rats. Vertical bars indicate standard errors. Values not sharing a common letter are significantly different at $p < 0.05$ by one-way analysis of variance followed by Fisher's protected least significant difference (PLSD) test. C, basal diet (control) group; YS, yellow soybean diet group; BS, black soybean diet group.

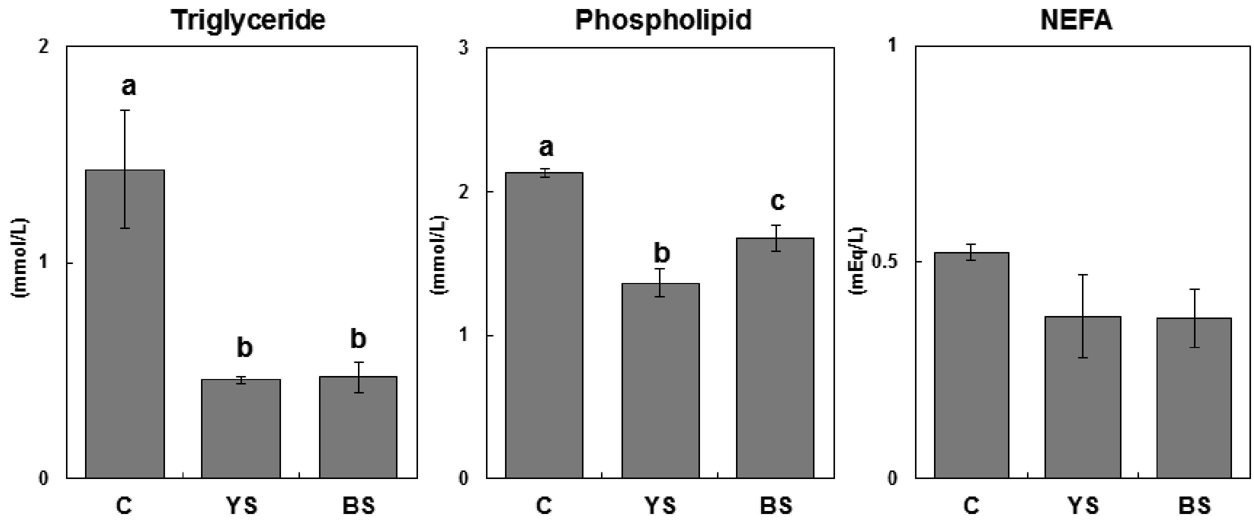

Fig. 2. Effects of the feeding of soybean on serum triglyceride, phospholipid, and nonesterified fatty acid (NEFA) concentrations in rats. Values represent the means for five rats. Vertical bars indicate standard errors. Values not sharing a common letter are significantly different at $p < 0.05$ by one-way analysis of variance followed by Fisher's protected least significant difference (PLSD) test. C, basal diet (control) group; YS, yellow soybean diet group; BS, black soybean diet group.

black soybean diet groups was significantly lower than that in the basal diet group, and it was significantly lower in the yellow soybean diet group than in the black soybean diet group. The serum NEFA concentration was not significantly different among the three diet groups.

Liver lipid contents are shown in Fig. 3. The liver cholesterol content was not significantly different among the three diet groups, although the level in the yellow and black soybean diet groups was lower but without a significant difference as compared to the basal diet group. The liver triglyceride content in the yellow and black soybean diet groups was significantly lower than that in the basal diet group. The liver phospholipid content was not significantly different among the three diet groups.

Serum and liver TBARS values are shown in Fig. 4. The liver TBARS value in the yellow and black soybean diet groups was significantly higher than that in the basal diet group, and this value was significantly lower in the black soybean diet group than in the yellow soybean diet group. The serum TBARS value was not significantly different among the three diet groups.

DISCUSSION

The feeding of yellow soybeans decreased the serum total cholesterol concentration in rats. With regard to lipoprotein cholesterol, the HDL-cholesterol concentration was decreased while the LDL- and VLDL-cholesterol concentrations were not changed by the feeding of yellow soybeans. The soybean has been demonstrated to have many nutritional functions that affect the cholesterol metabolism. In a previous study, a soybean protein isolate decreased the serum total cholesterol concentration, particularly the HDL-cholesterol concentration, in rats.[22] In the present study, rats were fed soybean whole beans that included a 20% protein fraction, so that the protein content was almost the same as in the above-mentioned study. The serum cholesterol-lowering effect seen in the present study might be due, at least in part, to the ingestion of the protein fraction of the soybean. The soybean protein isolate has been demonstrated to increase the excretion of neutral sterols[4] and bile acids[4,22] in the feces. The neutral sterols originate from cholesterol in the liver and serum, and the bile acids are biosynthesized from cholesterol in the liver. The increases in the neutral sterol and bile acid excretion in the feces lead to a reduction of the liver cholesterol content, resulting in an increase in the incorporation of cholesterol in blood; this may be why the serum cholesterol concentration was reduced by the feeding of soybeans. It is possible that the serum total and HDL-cholesterol concentration-lowering effects of the yellow soybean seen in the present study might be related, at least in part, to the increase in the excretion of neutral sterols and bile acids. Other investigators have demonstrated that the soybean protein isolate decreased the serum non-HDL-cholesterol ((VLDL+LDL)-cholesterol) concentration in rats.[4] In the present study, the LDL- and VLDL-cholesterol concentrations were not changed by the feeding of yellow soybeans. The feeding of the whole beans might weaken the serum non-HDL-cholesterol-lowering effect of the protein fraction.

The ingestion of both yellow and black soybeans decreased the serum triglyceride concentration and liver triglyceride content. The serum and liver triglyceride-lowering effects of the consumption of soybeans have been previously investigated.[5,23] The mechanisms of these serum and liver triglyceride-lowering effects are believed to include the following: the soybean protein suppresses the fatty acid

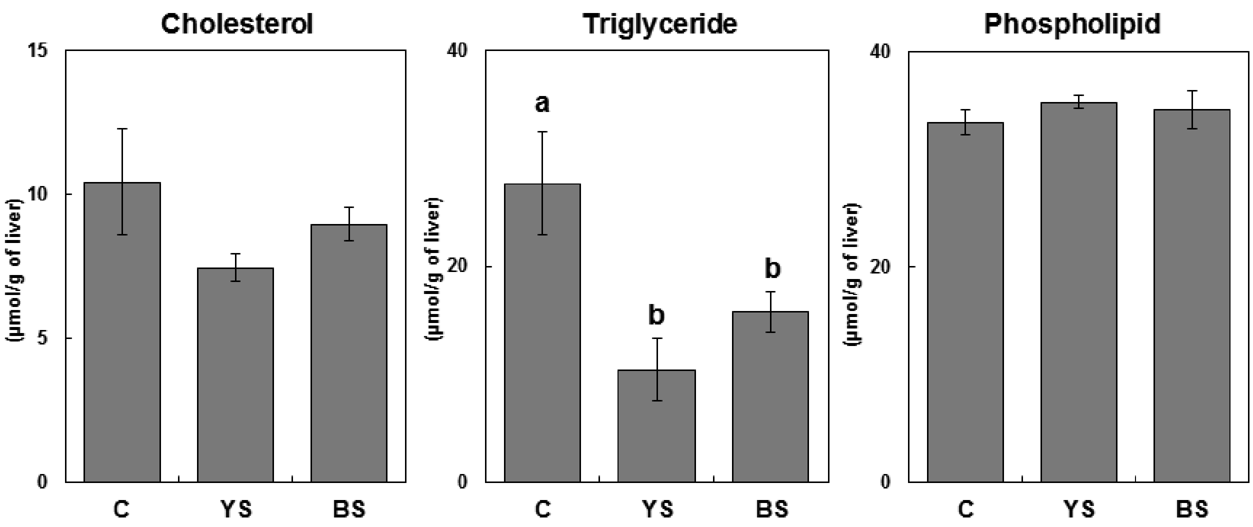

Fig. 3. Effects of the feeding of soybean on liver lipid contents in rats. Values represent the means for five rats. Vertical bars indicate standard errors. Values not sharing a common letter are significantly different at $p < 0.05$ by one-way analysis of variance followed by Fisher's protected least significant difference (PLSD) test. C, basal diet (control) group; YS, yellow soybean diet group; BS, black soybean diet group.

synthase,[23] triglyceride secretion from the liver to the blood is suppressed due to an enhancement of liver fatty acid β-oxidation,[5] and triglyceride excretion in the feces is increased.[23] Soybean protein is considered to reduce triglyceride anabolism while enhancing triglyceride catabolism; this may be why the serum triglyceride concentration and liver triglyceride content were decreased by the feeding of both yellow and black soybeans in the present study.

The liver TBARS in the black soybean diet group was significantly lower than that in the yellow soybean diet group. The black soybean contains a pigment in its seed coat that is mainly proanthocyanidin, a polyphenolic compound. The proanthocyanidin is a natural and water-soluble antioxidant that has great potential to scavenge oxygen free radicals, resulting in strong antioxidative action.[24] The TBARS value is an index of lipid peroxidation in the blood or tissues. The degree of fatty acid peroxidation in the liver is likely to be suppressed by the polyphenolic compounds including proanthocyanidin in the black soybean; this may be why the TBARS value in the liver was decreased by the black soybean diet as compared to the yellow soybean diet.

Body weight gain was significantly decreased by the feeding of

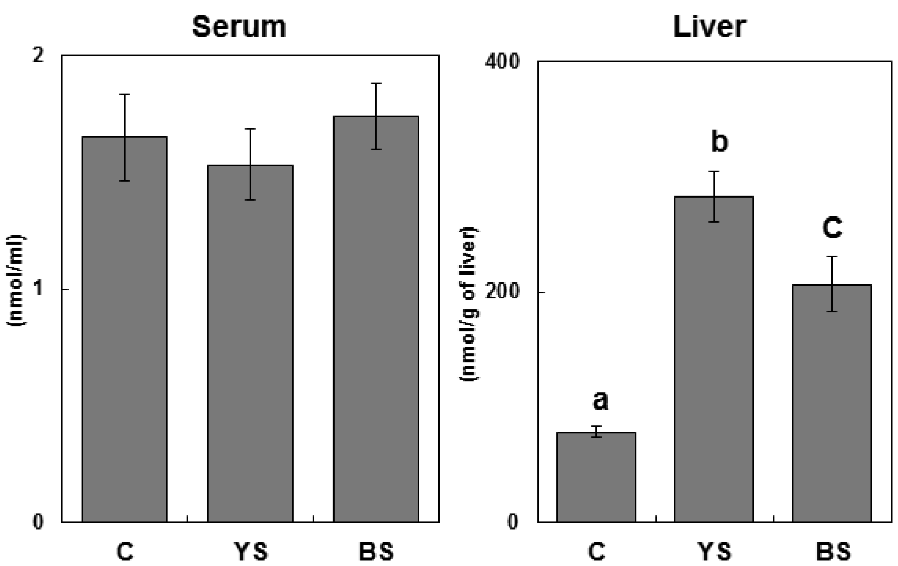

Fig. 4. Effects of the feeding of soybean on serum and liver thiobarbituric acid-reactive substance (TBARS) values in rats. Values represent the means for five rats. Vertical bars indicate standard errors. Values not sharing a common letter are significantly different at $p < 0.05$ by one-way analysis of variance followed by Fisher's protected least significant difference (PLSD) test. C, basal diet (control) group; YS, yellow soybean diet group; BS, black soybean diet group.

both yellow and black soybeans as compared to the control group in spite of the absence of a significant difference in food intake among the three groups. In the present study, both the yellow and black soybeans used in the experimental diets were raw. The soybean is known to contain a trypsin inhibitor which prevents protein digestion; resulting in the inhibition of protein absorption. This disorder in protein absorption may result in malnutrition based on a lack of protein synthesis accompanied by a decrease in the supply of amino acids from dietary protein. Protein mainly constitutes the muscle and bone in the body, which account for the majority of body weight; this may be why the decrease in body weight gain due to the feeding of soybeans was investigated in the present study.

In conclusion, the feeding of soybeans has beneficial effects on the lipid metabolism. These effects have been shown not only for animals[4-7,22,23] but also for humans.[25] Furthermore, the consumption of soybeans also has beneficial effects on other diseases.[8-11] In addition to these findings, it was shown that black soybeans had an antioxidative effect resulting from the suppression of the liver TBARS value. These findings suggest that the consumption of soybeans has health benefits; thus, it is recommended to incorporate these soybeans into the daily diet.

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和文要旨　大豆摂取の血清および肝臓脂質レベルに対する影響をラットにおいて検討した。ラットには黄大豆ならびに黒大豆を含む飼料を，基本飼料とタンパク質，脂質ならびに食物繊維量が同等の条件下で与えた。血清総コレステロール濃度が，黄大豆を含む飼料の摂取により基本飼料摂取と比べて有意に低下した。リポタンパク質ごとでは，高密度リポタンパク質コレステロール濃度が，黄大豆を含む飼料の摂取により基本飼料摂取と比べて有意に低下した。超低密度リポタンパク質コレステロール濃度ならびに低密度リポタンパク質コレステロール濃度に大豆摂取による有意な違いはみられなかった。肝臓コレステロール含量が，黄大豆ならびに黒大豆を含む飼料の摂取により有意な違いはみられなかったが，基本飼料摂取と比べて低下の傾向を示した。血清ならびに肝臓トリグリセリドレベルが，黄大豆ならびに黒大豆を含む飼料の摂取により基本飼料摂取と比べて有意に低下した。肝臓チオバルビツール酸反応物質値が，黄大豆ならびに黒大豆を含む飼料の摂取により基本飼料摂取と比べて有意に増加したが，黒大豆を含む飼料の摂取により黄大豆を含む飼料摂取と比べて有意に減少した。黄大豆ならびに黒大豆の摂取は，脂質代謝に対して有用な効果のあることが見出され，日常の食事において摂取していくことが推奨される。